



0999430.011303 #15

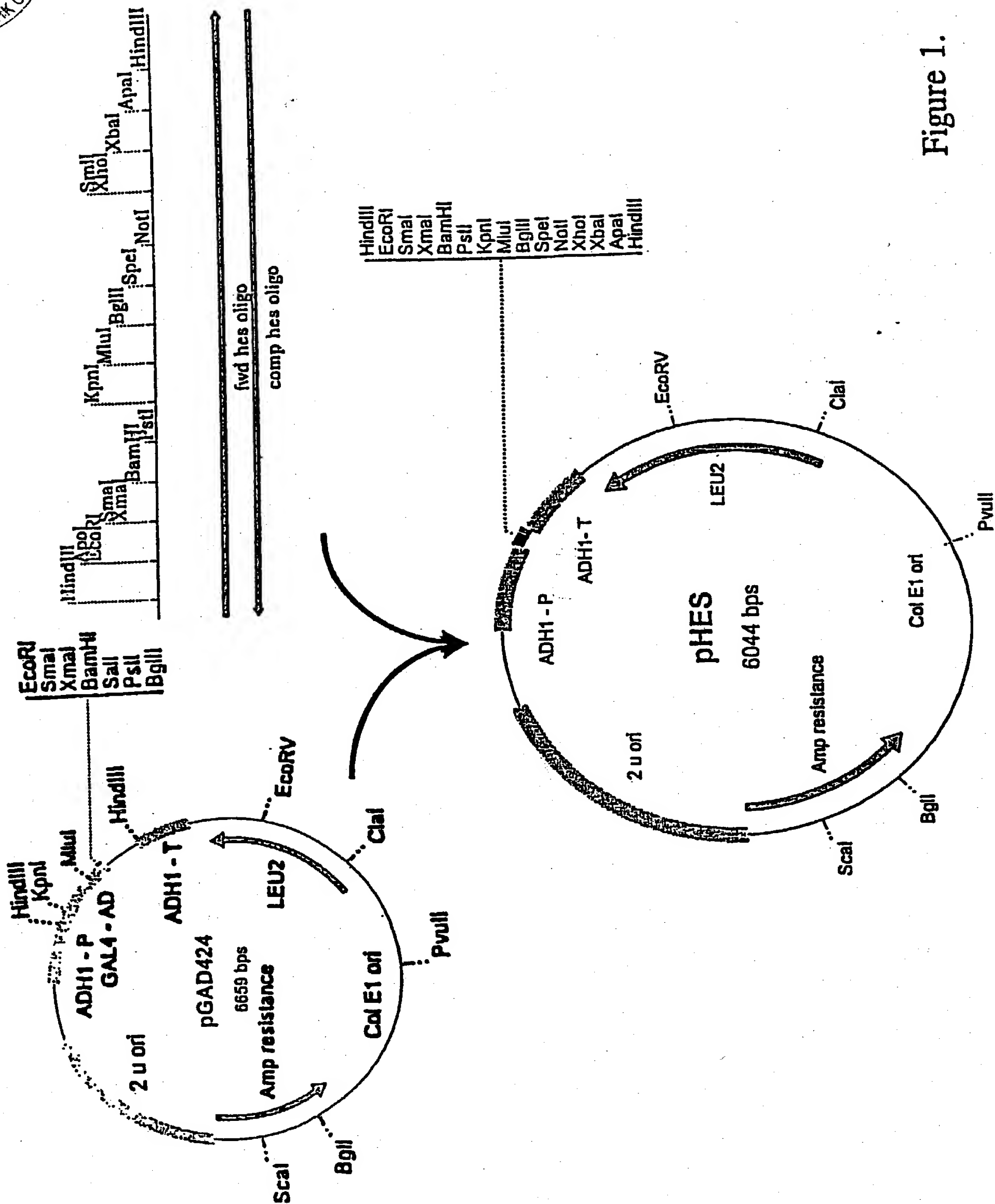


Figure 1.

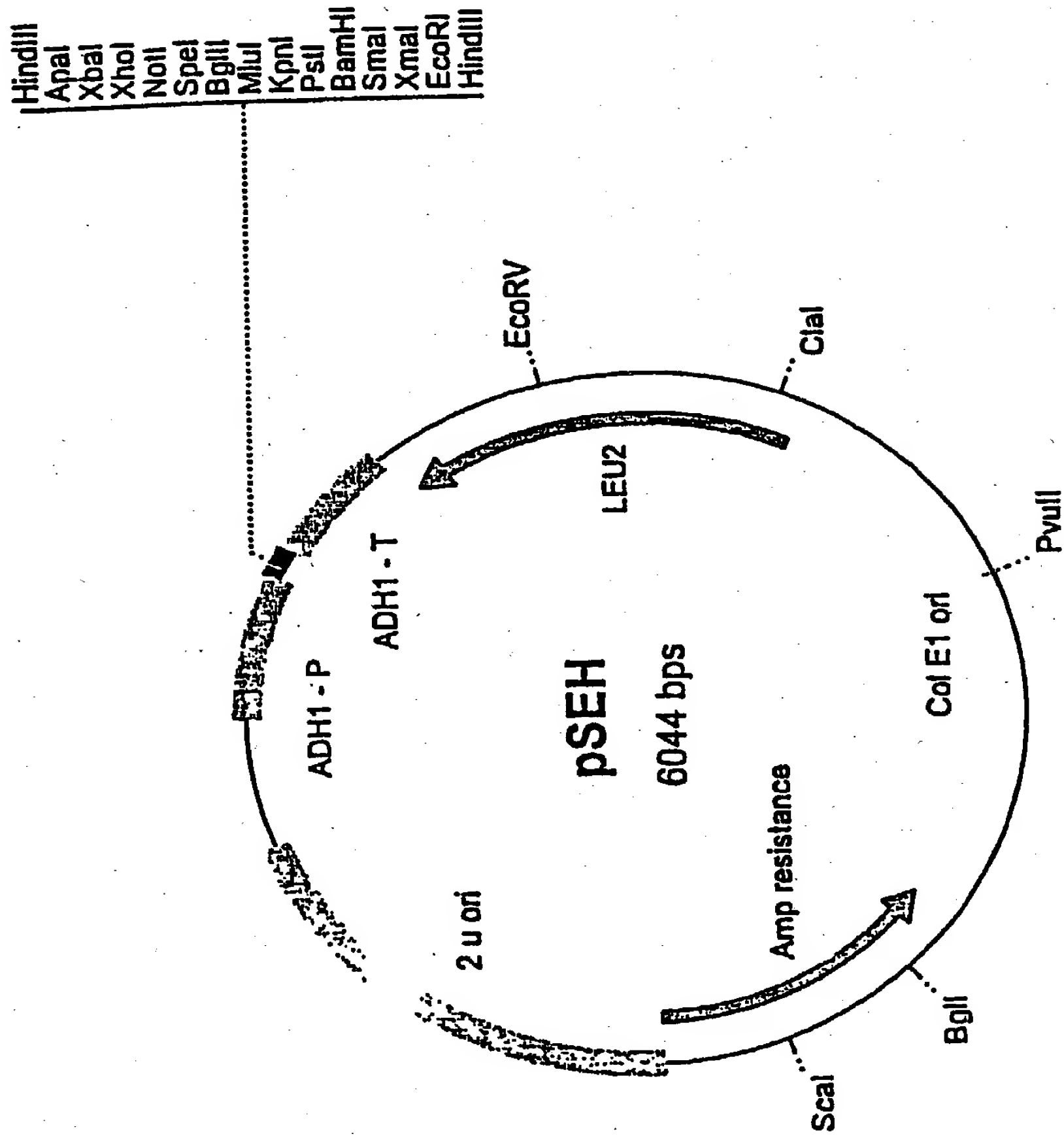


Figure 2.

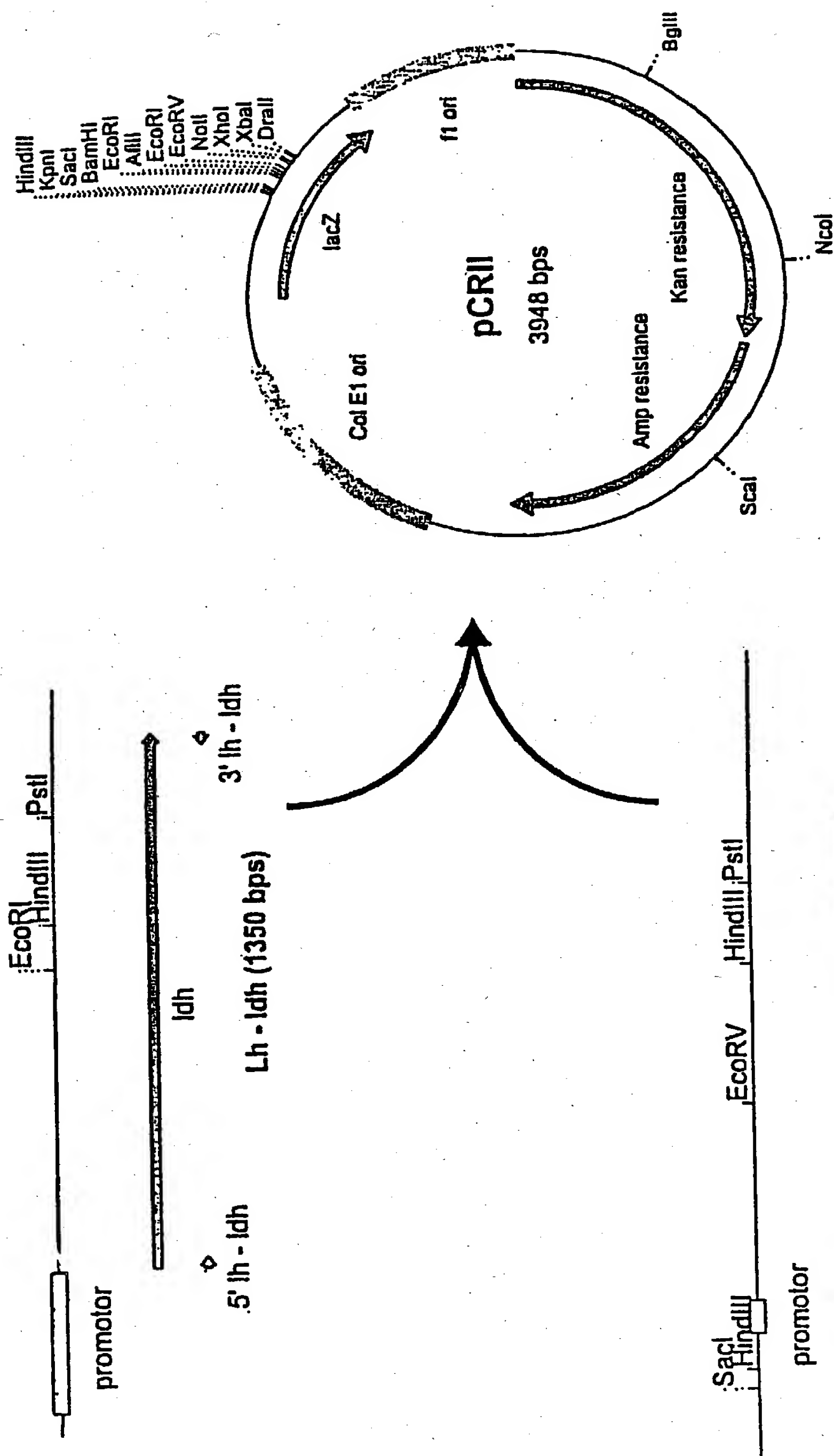


Figure 3.



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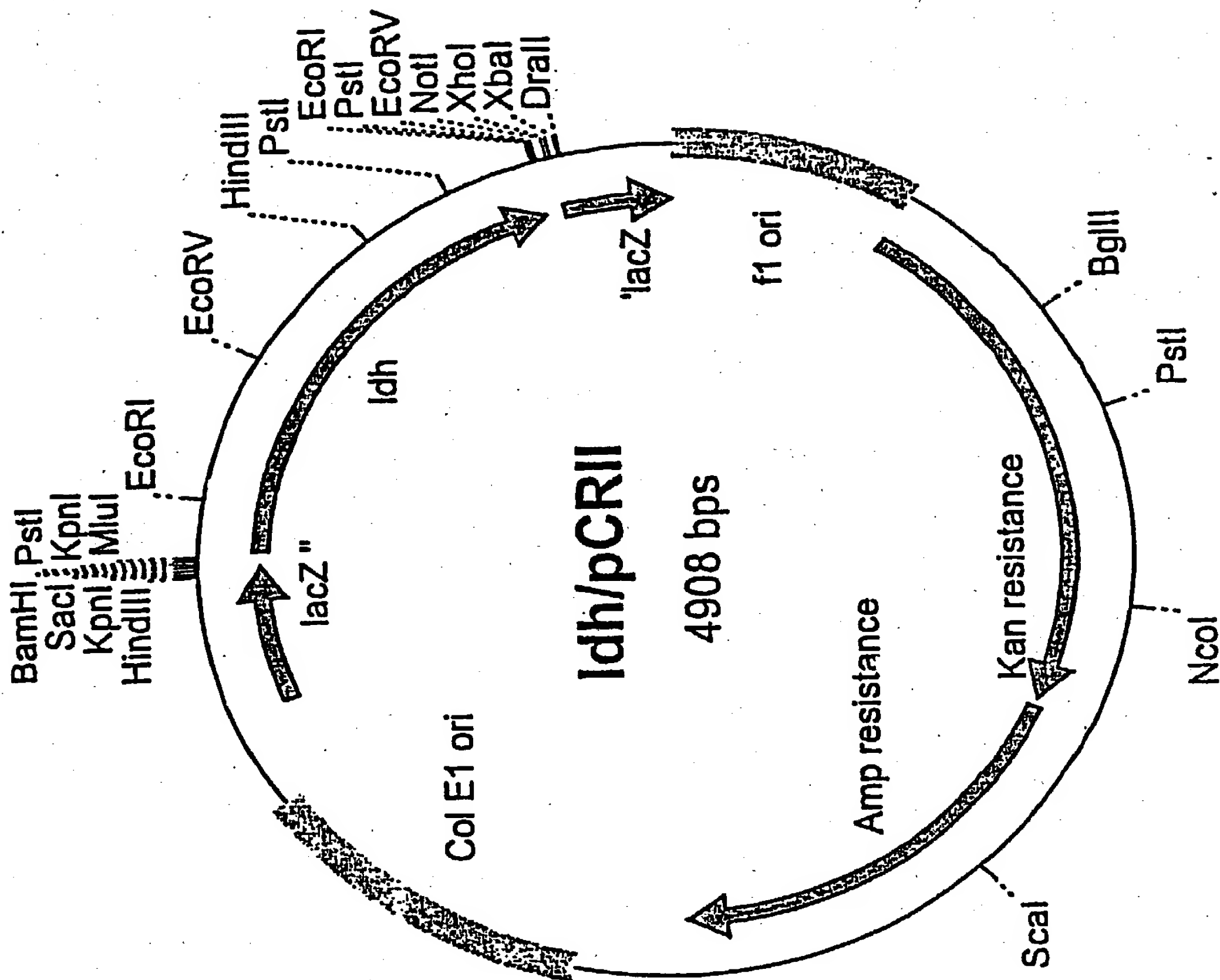


Figure 4.



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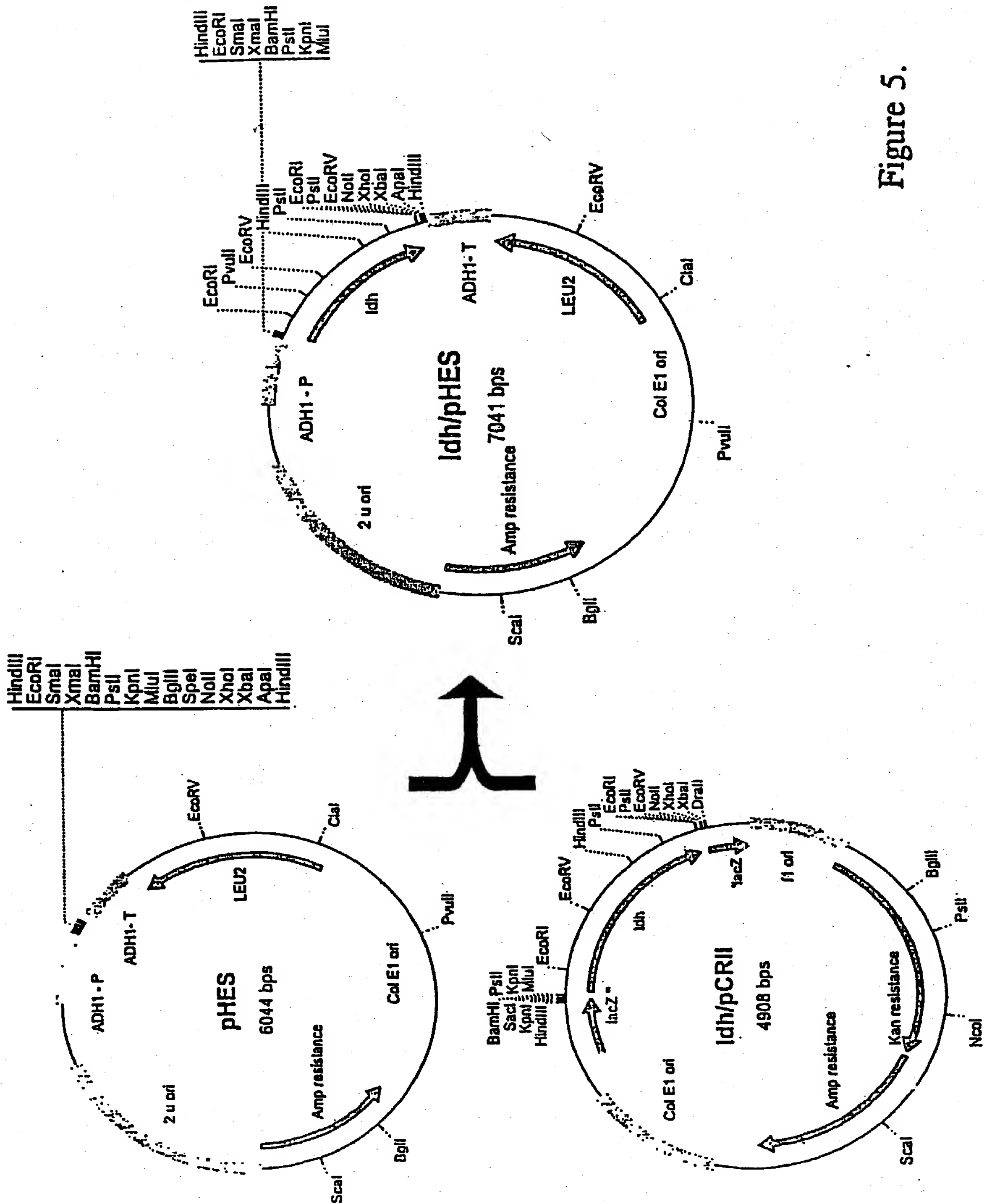


Figure 5.



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Figure 6b.

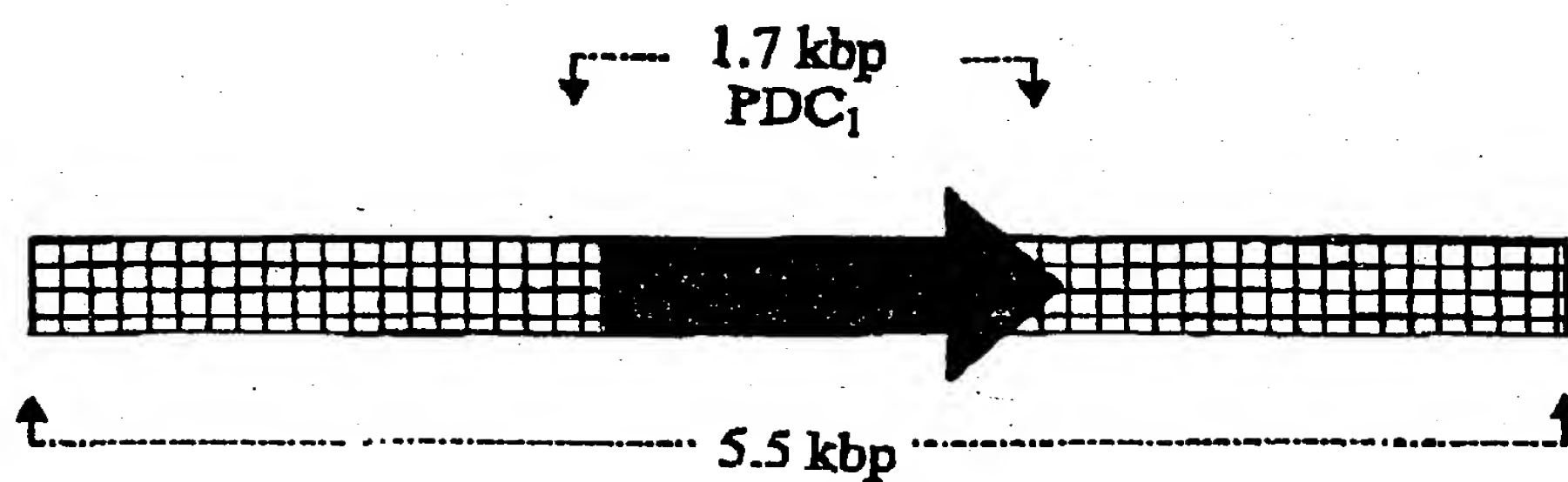




Figure 6c.

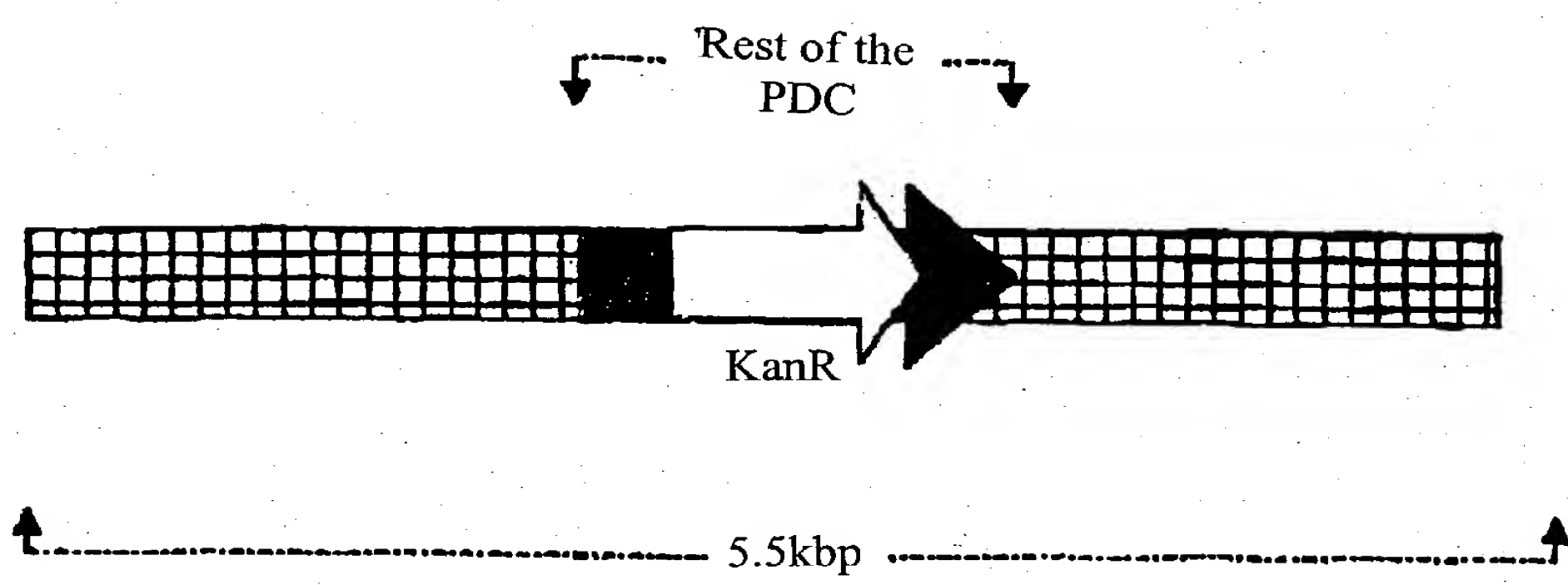
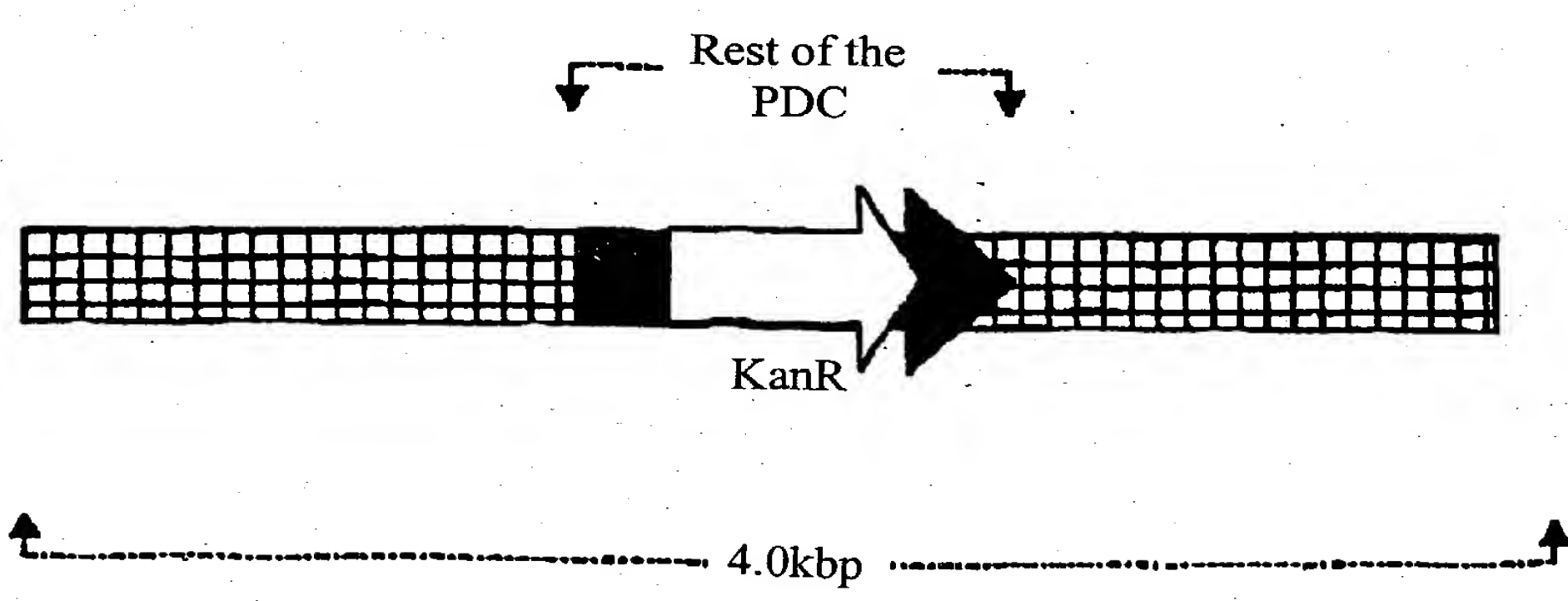


Figure 6d.



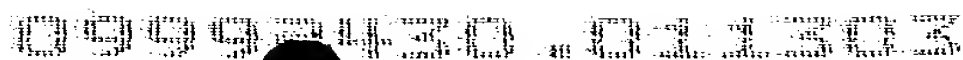


Figure 6e.

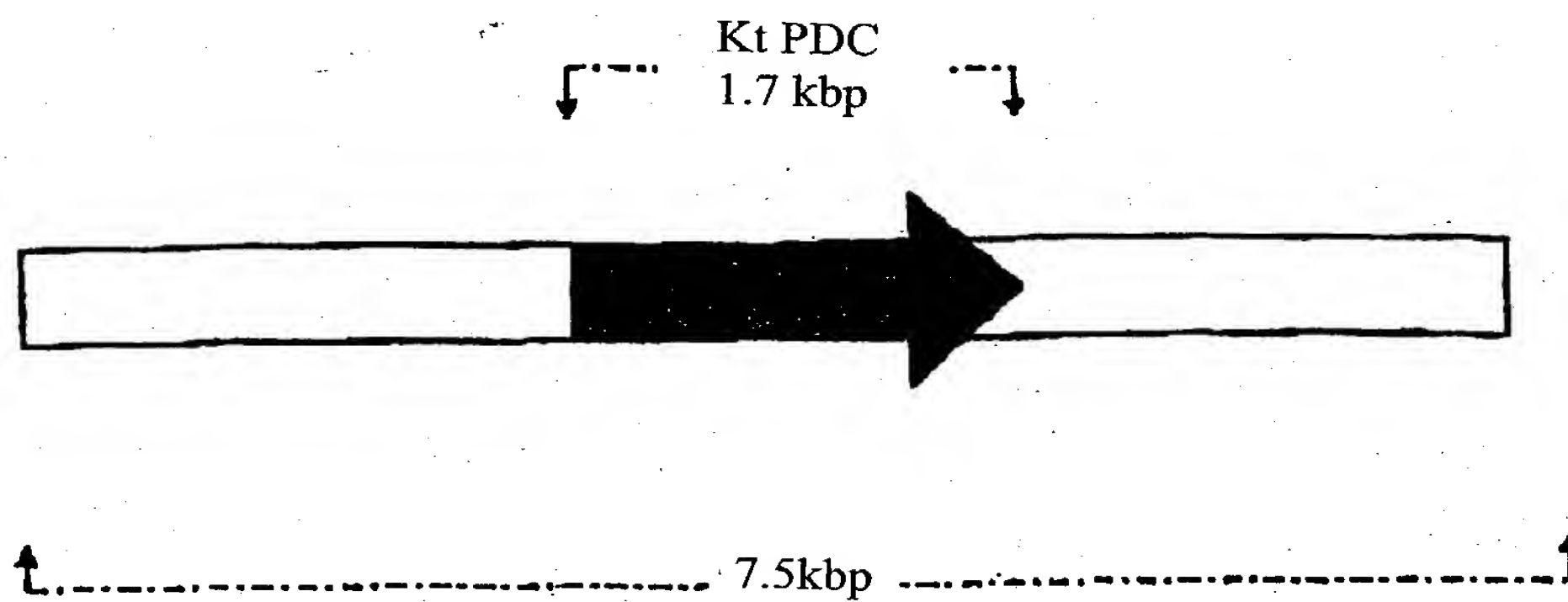
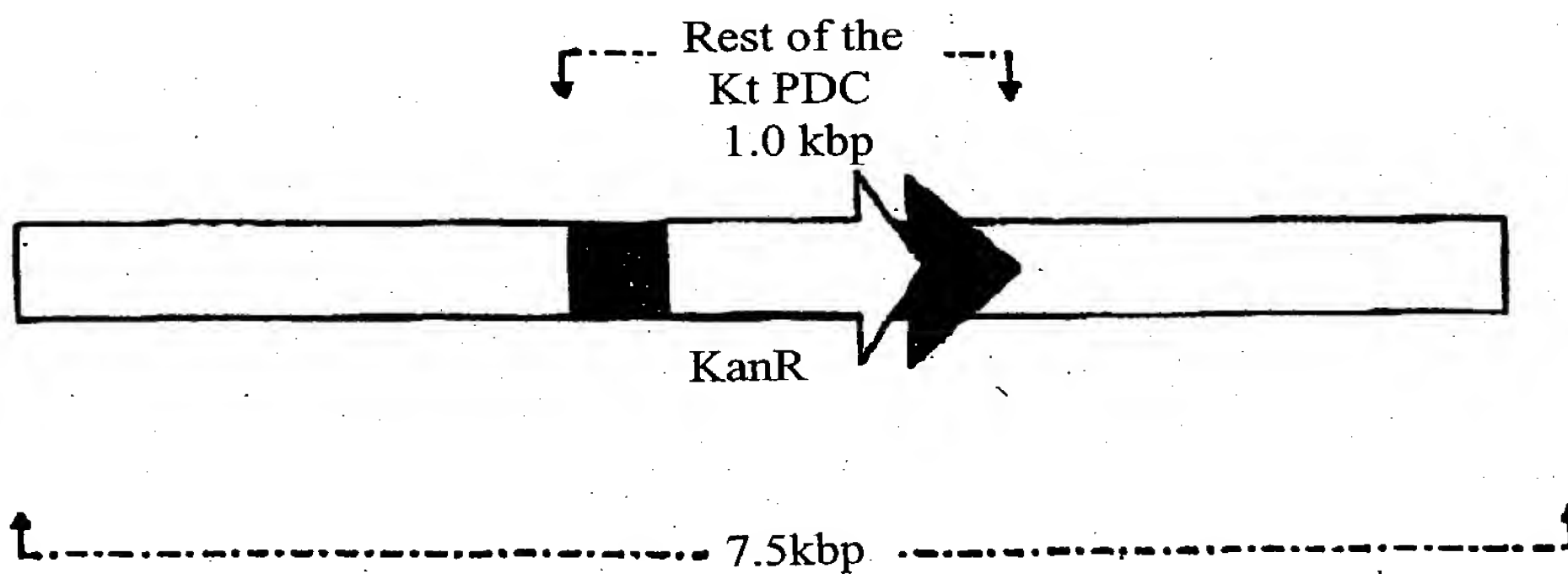


Figure 6f.







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Figure 7.

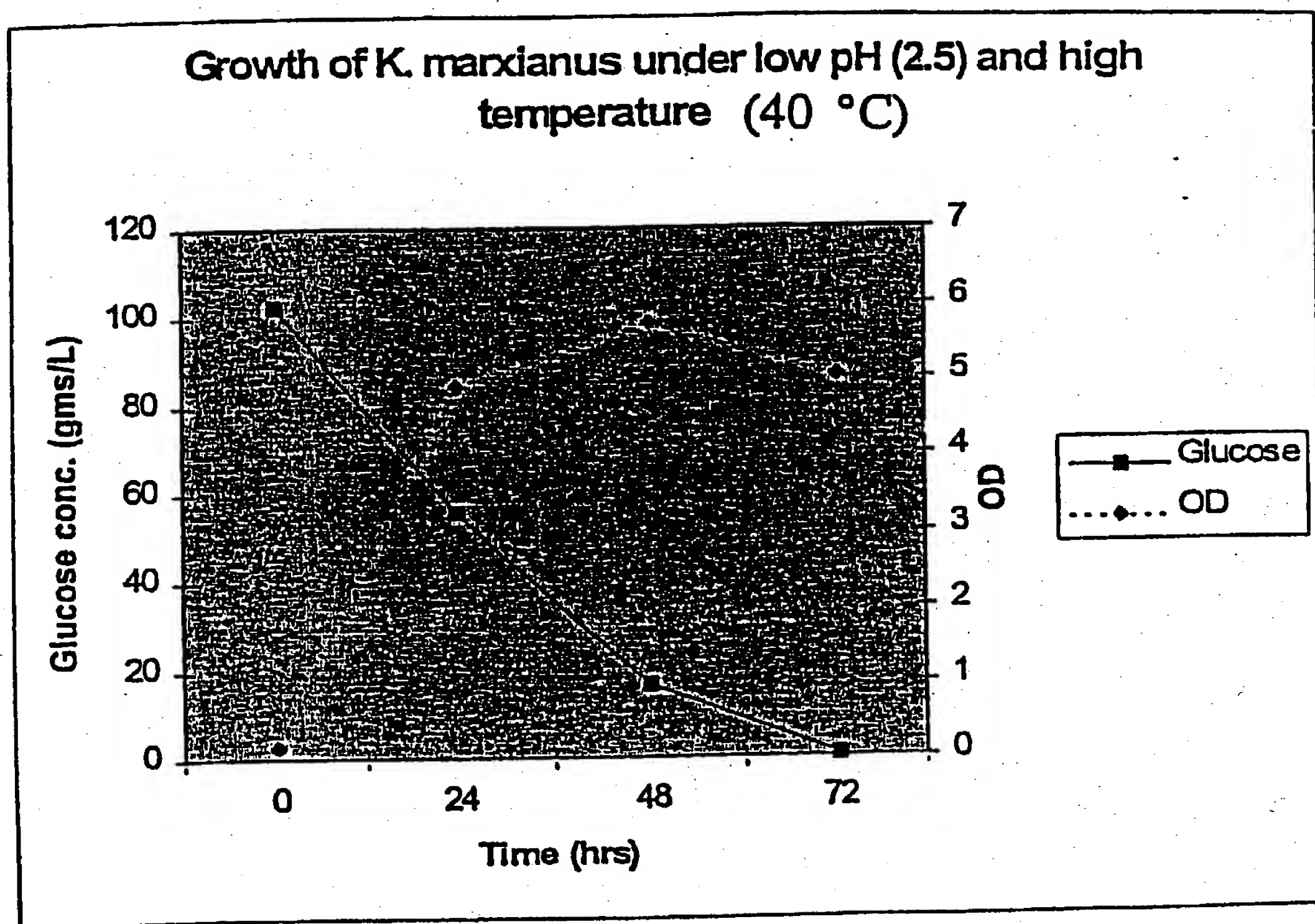
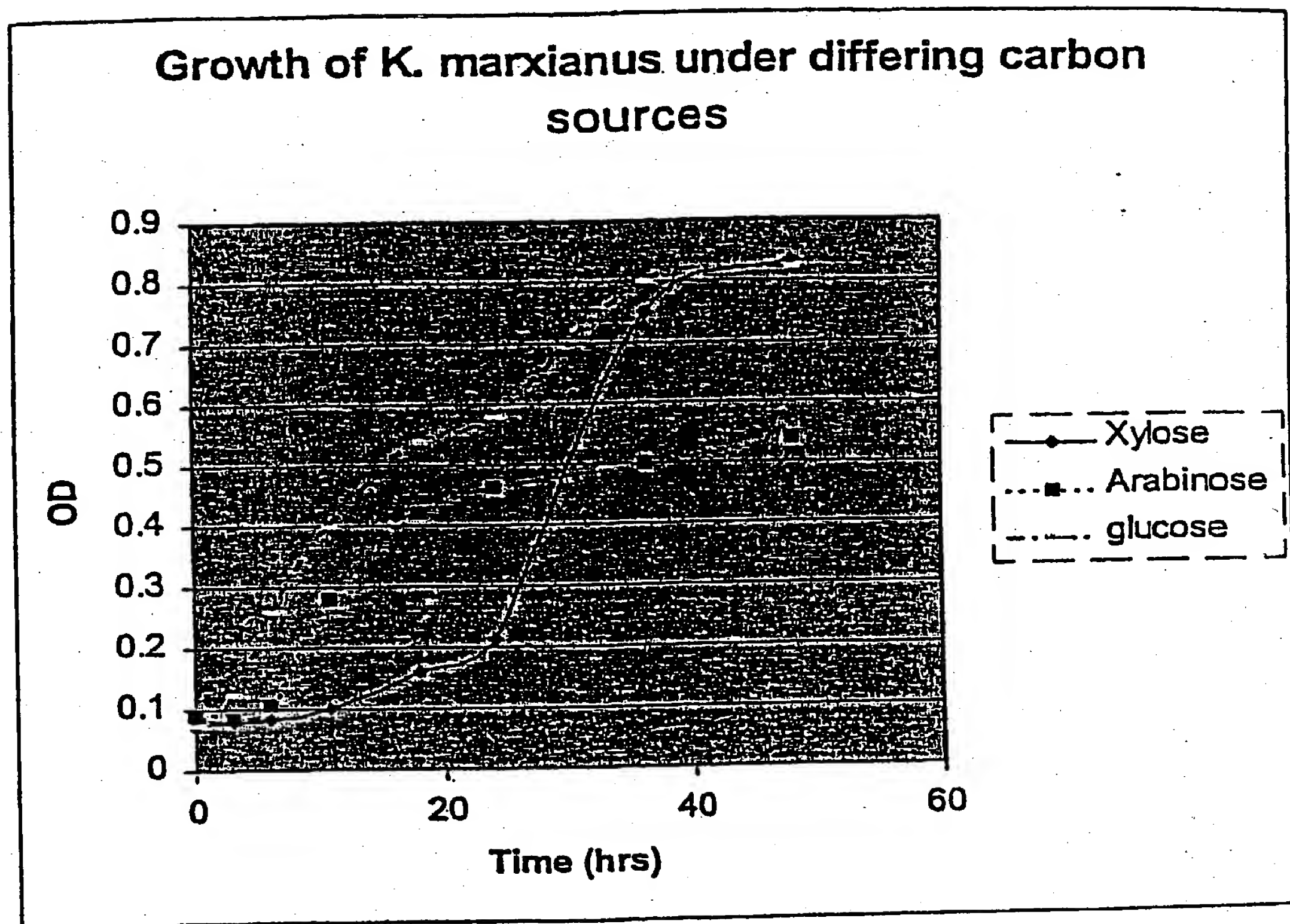




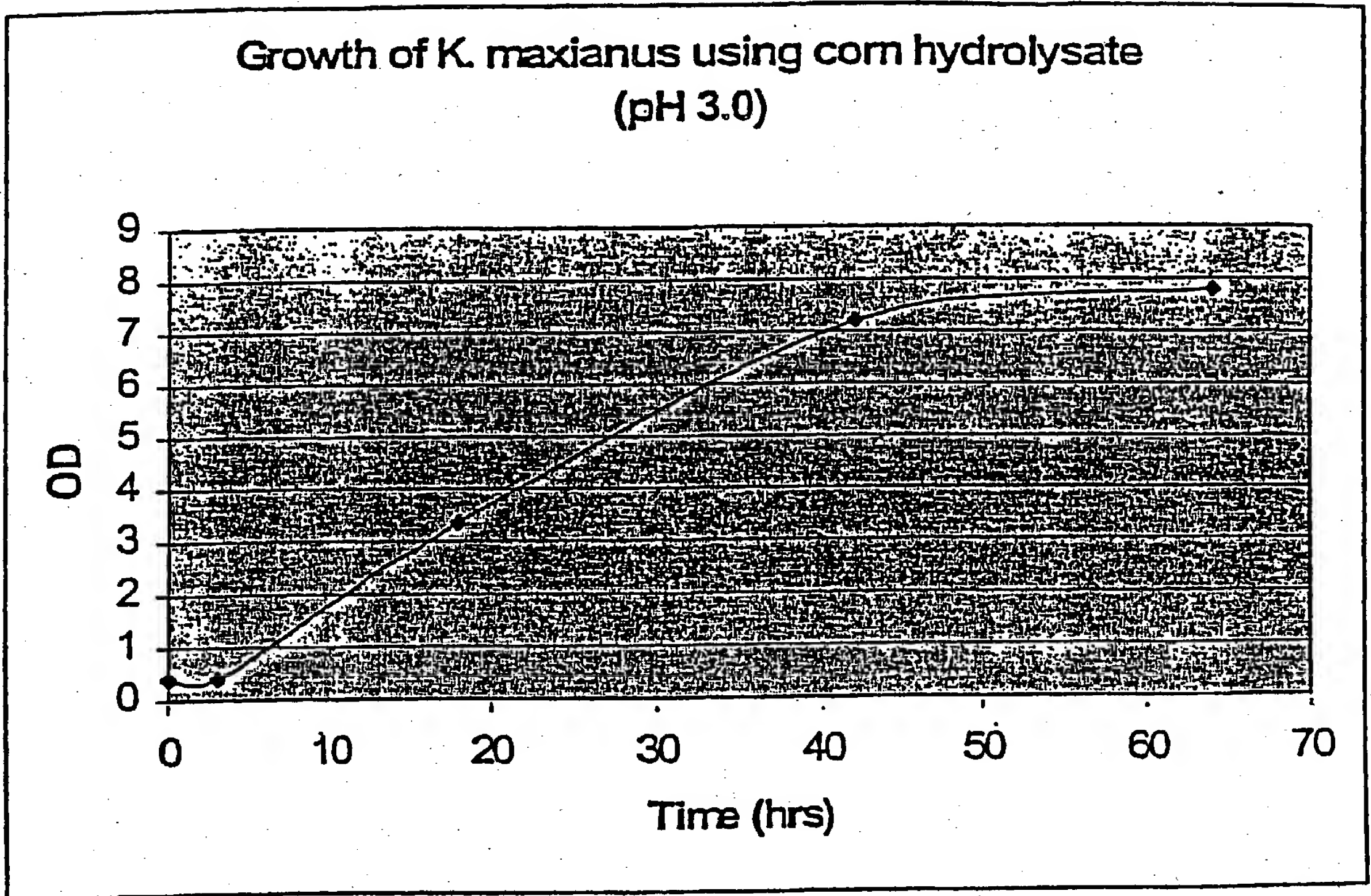
Figure 8.

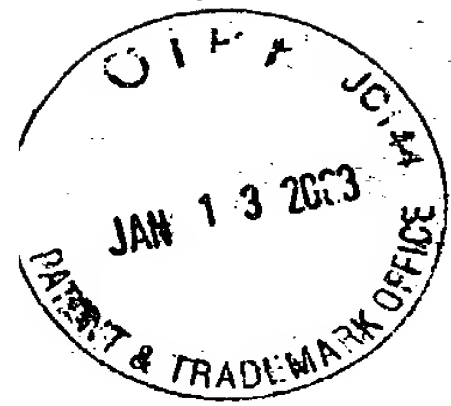




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Figure 9.





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# Growth curve for *K. marxianus* (pH adjusted using HCL-inorganic acid)

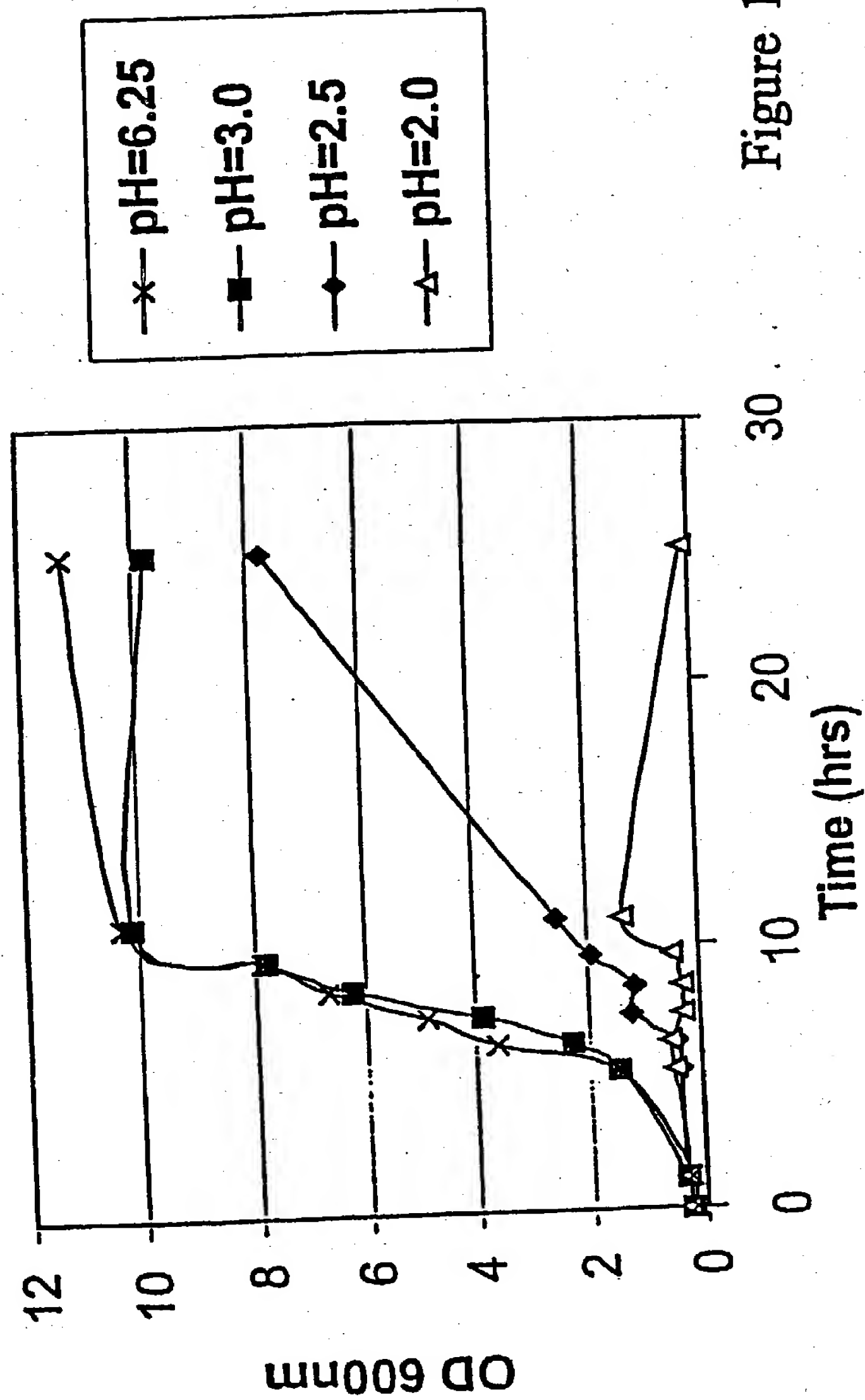


Figure 10.

# Growth curve for *K. marxianus* under low pH and 40gm/L lactic acid

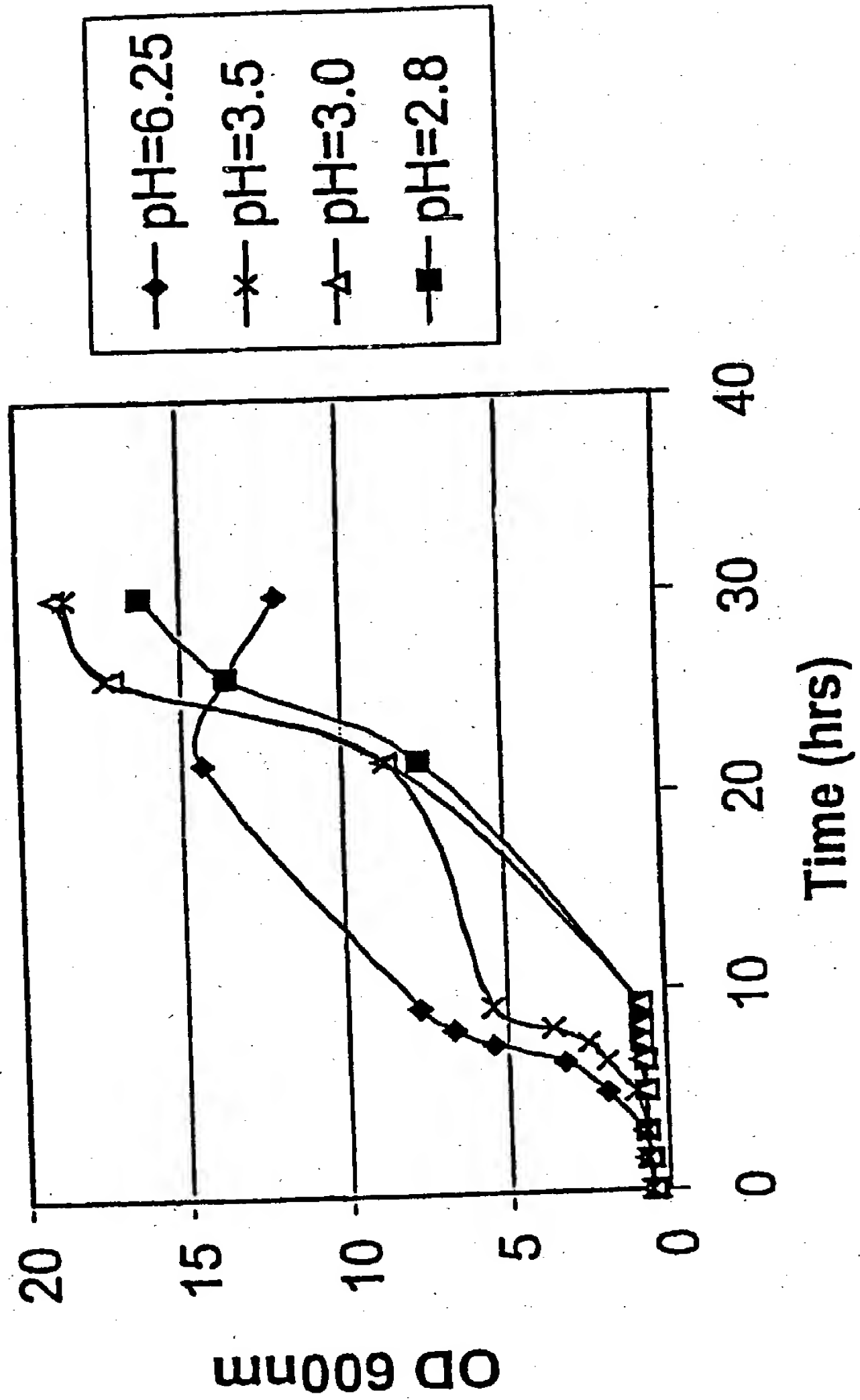


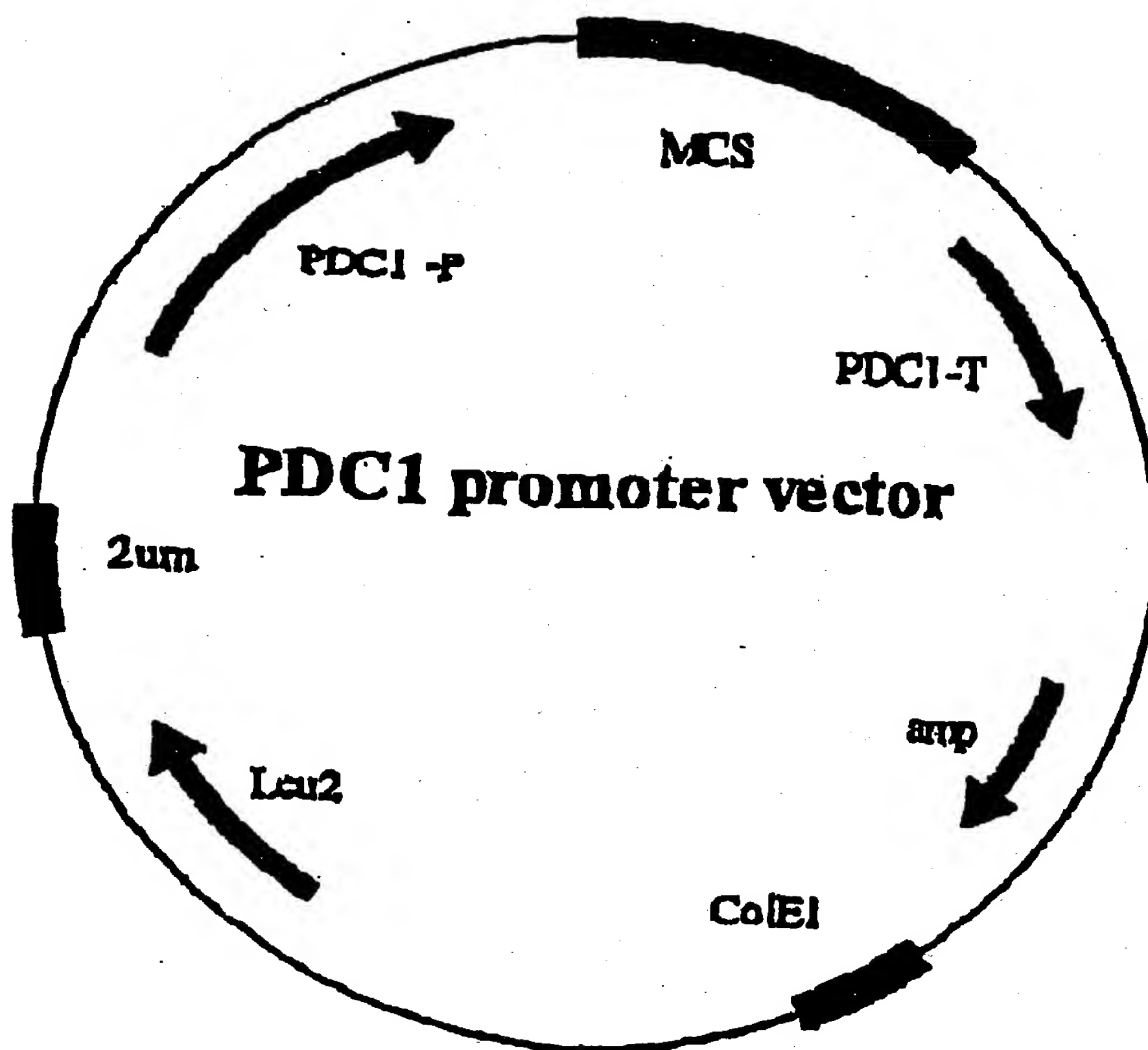
Figure 11.



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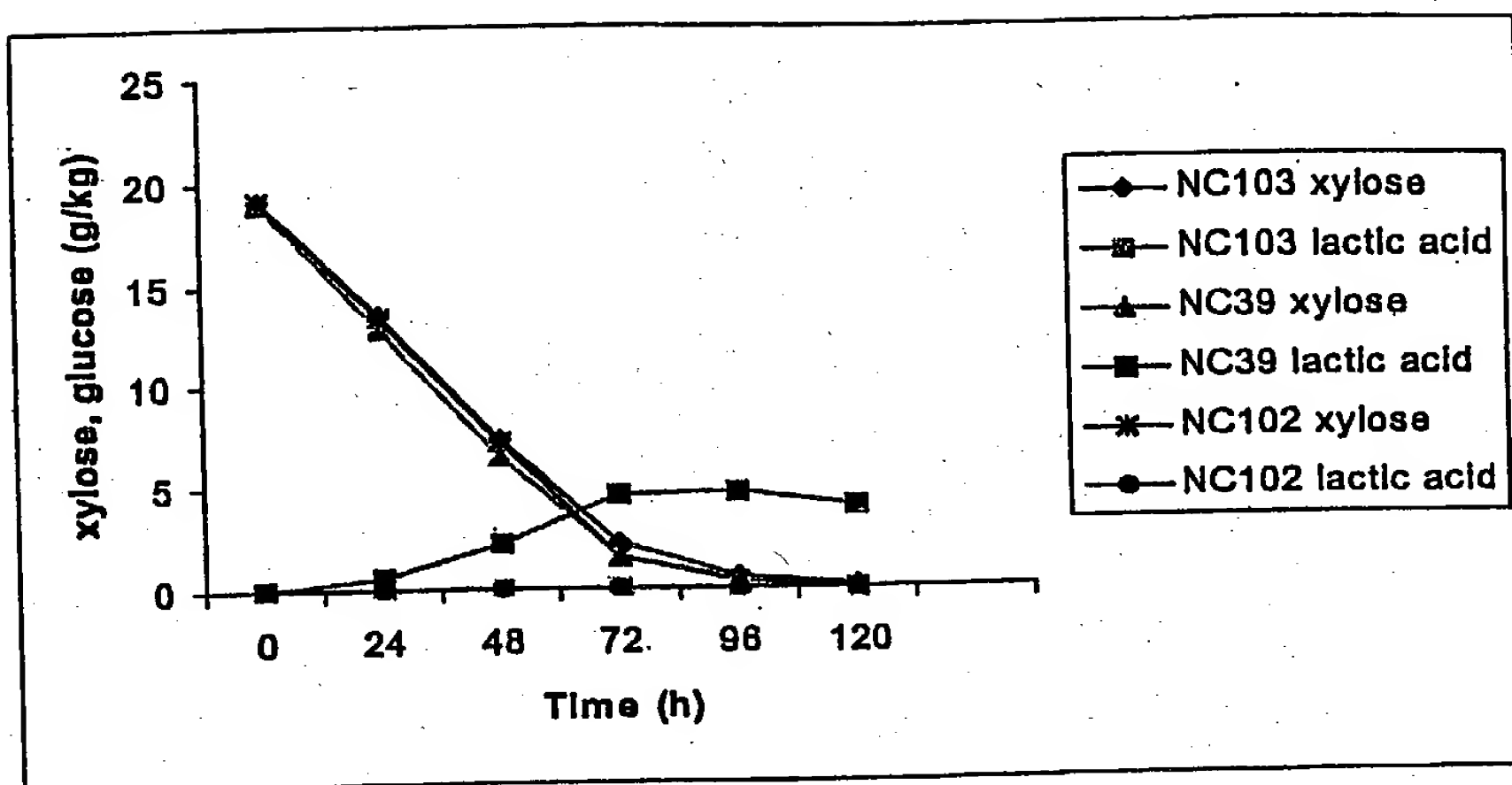
Figure 14





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Figure 15



A circular map of the pMI233 plasmid, which is 5097 base pairs (bps) in size. The map displays various restriction enzyme sites around the circumference, each labeled with the enzyme name and its position in base pairs. Key features include the *amp* (ampicillin resistance) gene, the *lacZ* gene, and the MEL5 (multiple cloning site). The map is divided into segments by these sites, with some segments labeled with their size in base pairs (e.g., 1000, 2000, 3000, 4000). The plasmid is shown with a thick black line representing the DNA backbone, and the restriction sites are indicated by short lines pointing to the map.

**Restriction Enzyme Sites (Clockwise from top):**

- BstUI, 5
- BstUI, 29
- BstUI, 49
- NaeI, 129
- NgoMIV, 129
- BsaAI, 232
- Drall, 232
- PsiI, 360
- BstUI, 425
- PvuII, 527
- EaeI, 609
- BstUI, 620
- BstUI, 622
- AccI, 710
- AccI, 797
- PsiI, 828
- BspHI, 861
- BsaI, 933
- BstXI, 1005
- EaeI, 1014++
- PsiI, 1053++
- HincII, 1133
- BpmI, 1353
- BtrI, 1451
- XbaI, 1545
- StuI, 1799
- HincII, 1907++
- KpnI, 1988
- TatI, 1981
- BstXI, 2050
- AhdI, 2112
- XbaI, 2187
- PvuII, 2229
- BsaI, 2255
- NspI, 2309
- SphI, 2309
- AccI, 2504
- AatII, 2507
- BsaHI, 2507
- 2569, BbsI
- 2671, BssSI
- 2678, NsiI
- 2678, Ppu10I
- 2692, NspI
- 2785, HincII
- 2816, BsaI
- 2929, BstUI
- 3111, PvuII
- 3128, EaeI
- 3135, BstUI
- 3137, BstUI
- 3166, SapI
- 3289, AflIII
- ++3289, NspI
- 3335, BstUI
- 2891, KpnI
- Acc65I
- PspOMI
- Apal
- EcoO109I
- XhoI
- Sall
- HincII
- AccI
- Clal
- HindIII
- EcoRV
- EcoRI
- 2843
- 3700, A1wNI
- 3916, BstUI
- 4009, BspHI
- 4177, AhdI
- 4246, BstUI
- 4249, BsaI
- 4287, BpmI
- 4570, EaeI
- 4680, Scal
- 4680, TatI
- 4718, BsaHI
- 4739, BstUI
- 4777, XmnI
- 4846, BssSI
- 5017, BspHI
- 5071, BstUI

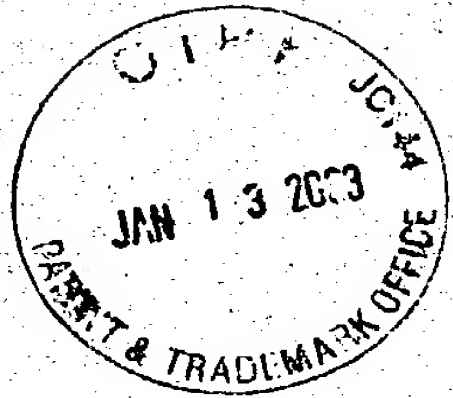
**Internal Features:**

- amp* (ampicillin resistance gene)
- lacZ* (lac operon)
- MEL5 (multiple cloning site)

**Segment Sizes (Clockwise from top):**

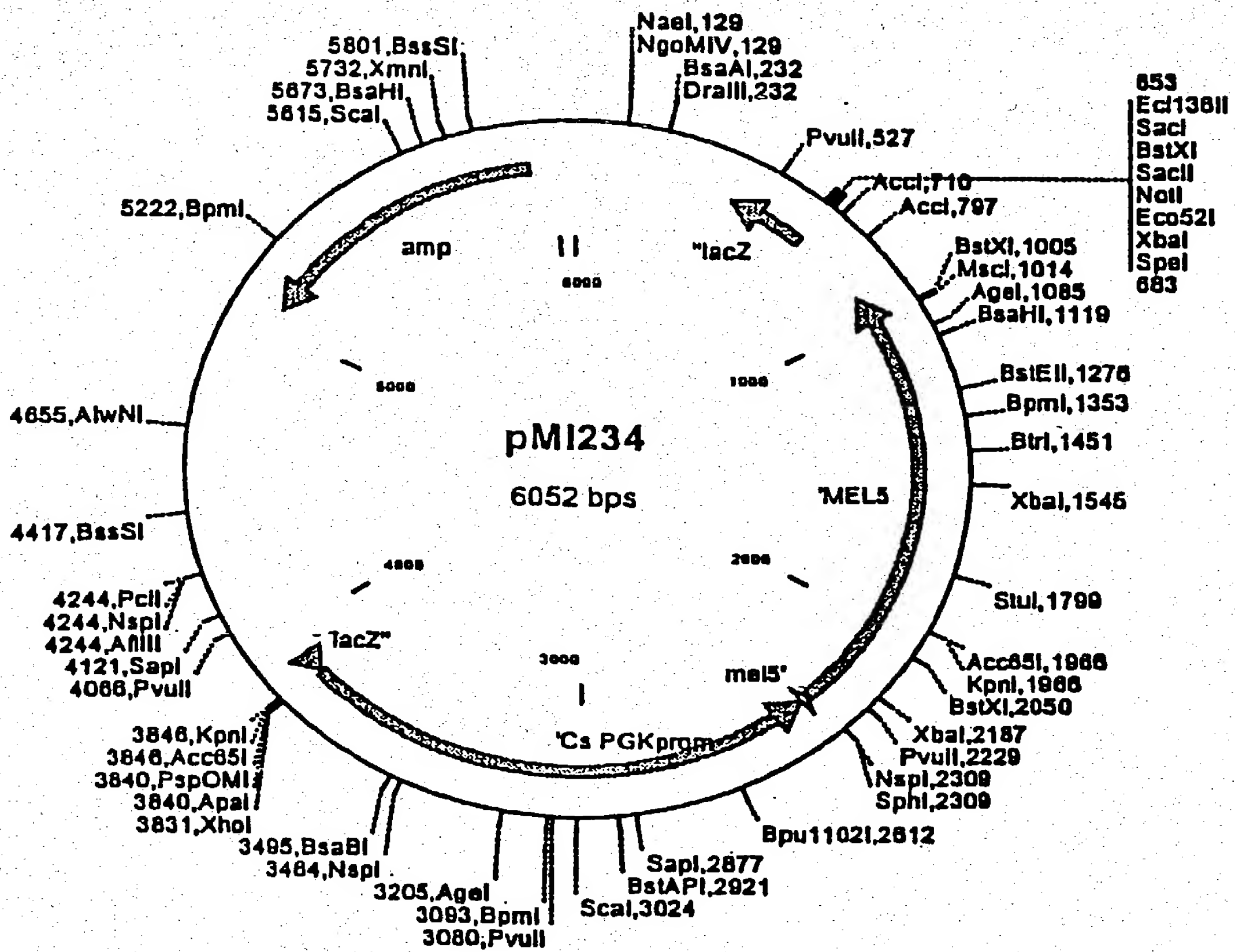
- 1000
- 1000
- 2000
- 3000
- 4000

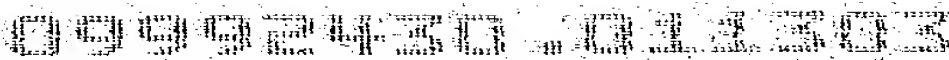




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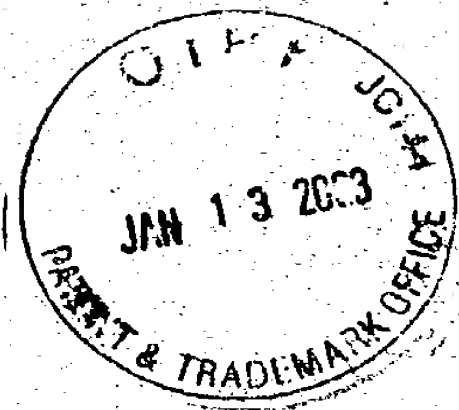
FIGURE 16 (2/11)



[illegible]

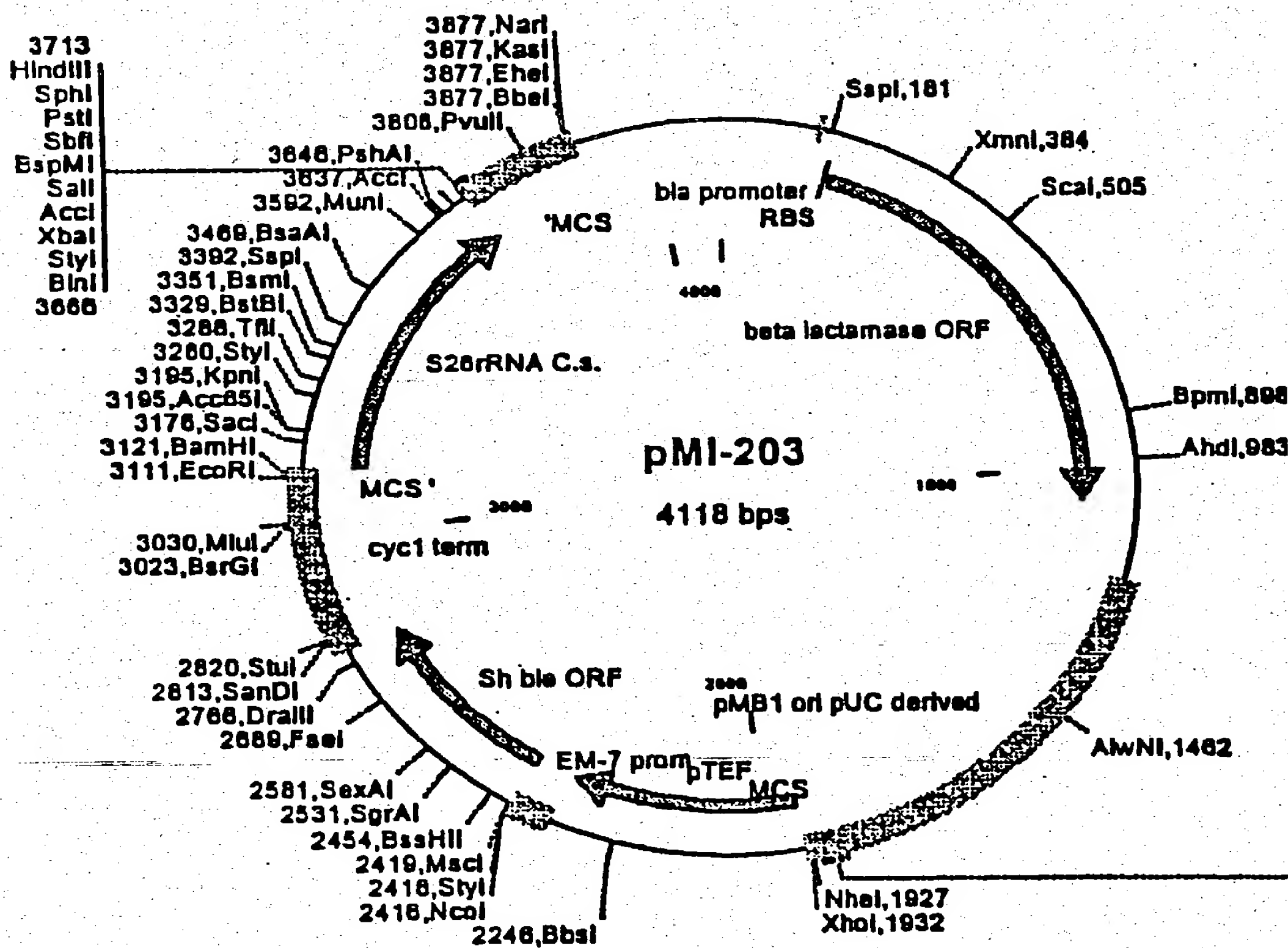
0147  
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 PATENT & TRADEMARK OFFICE

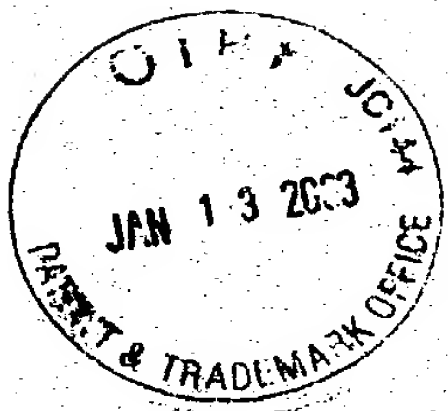
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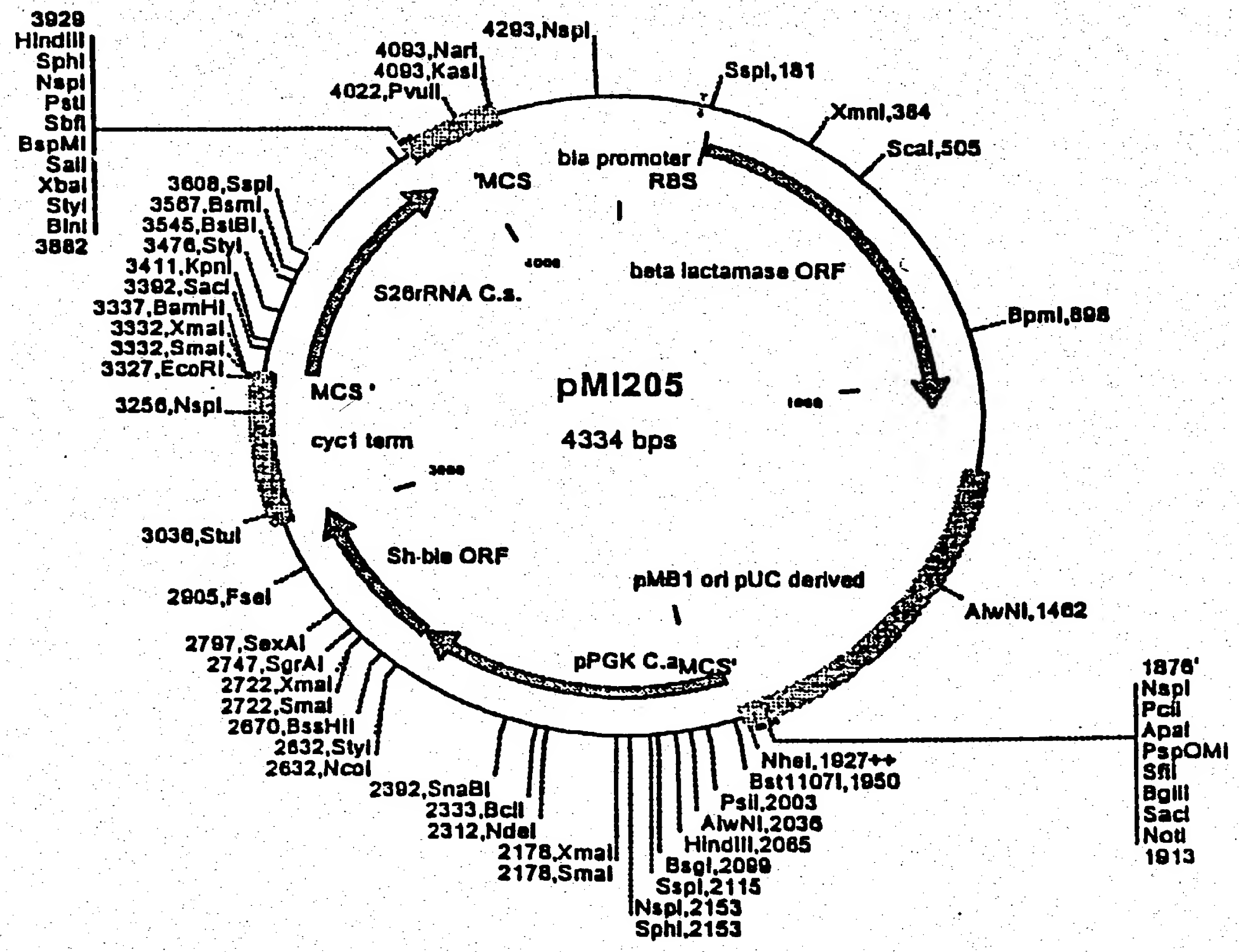
FIGURE 16 (5/11)





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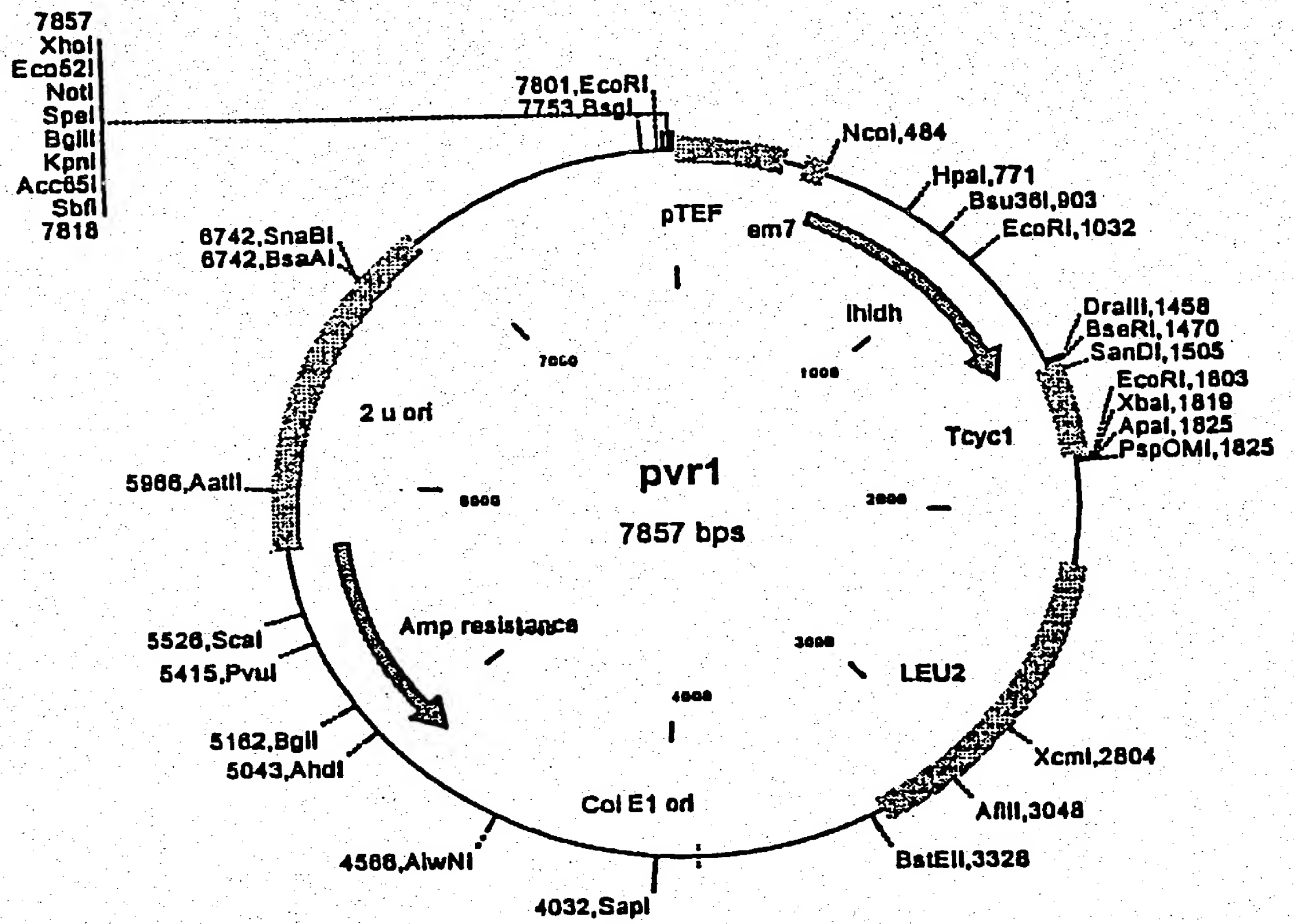
FIGURE 16 (6/11)





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FIGURE 16 (7/11)

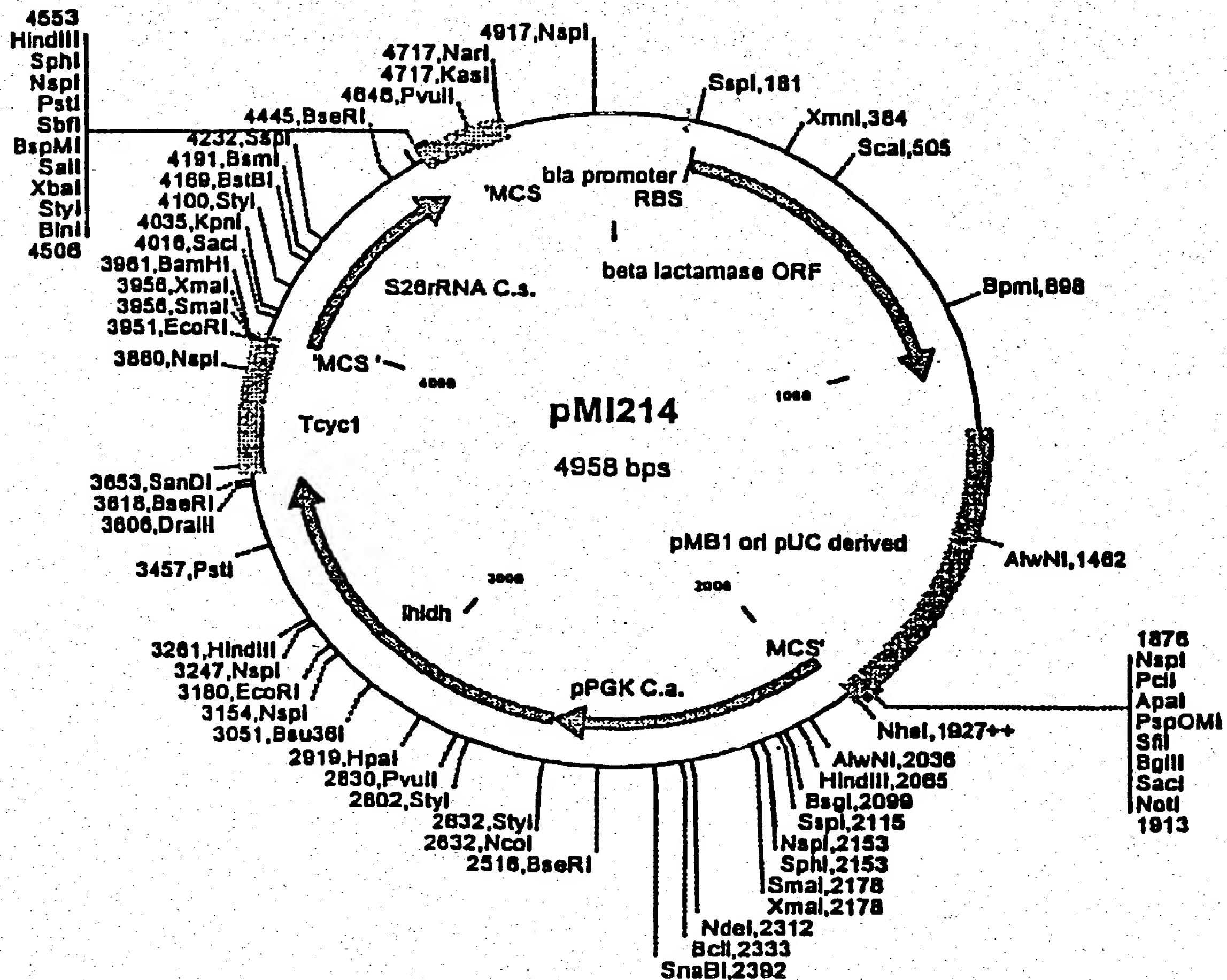






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FIGURE 16 (8/11)



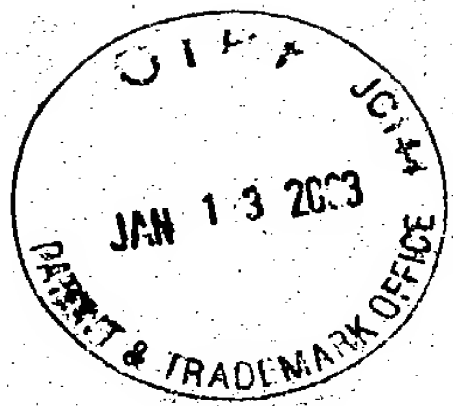
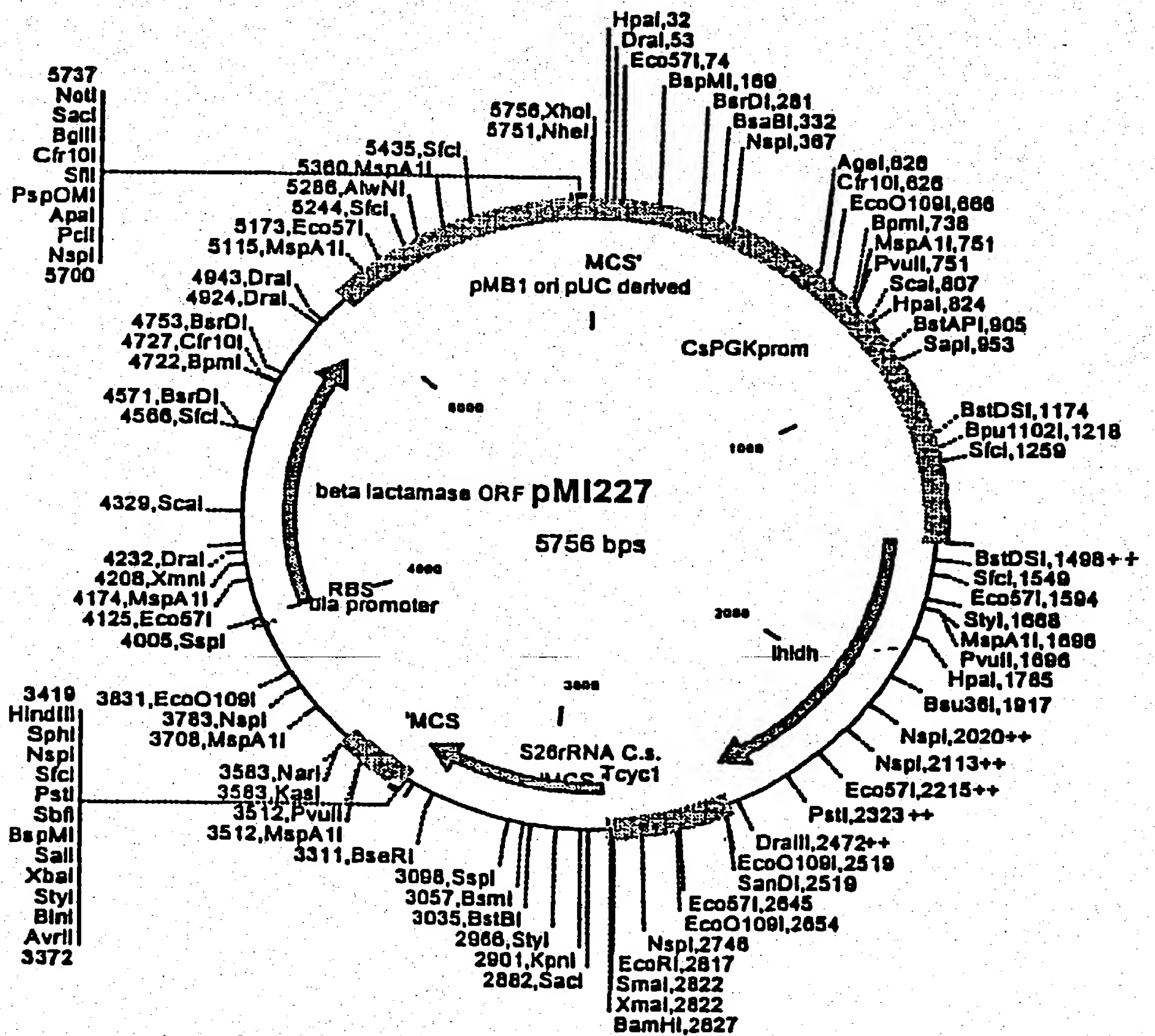


FIGURE 16 (9/11)





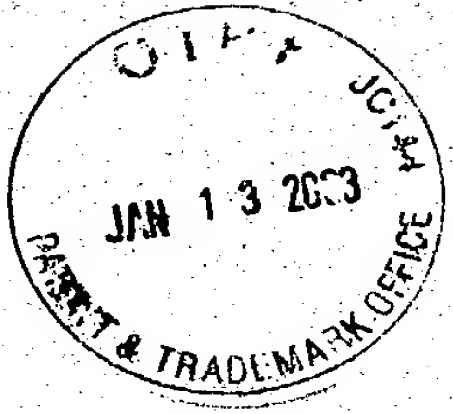
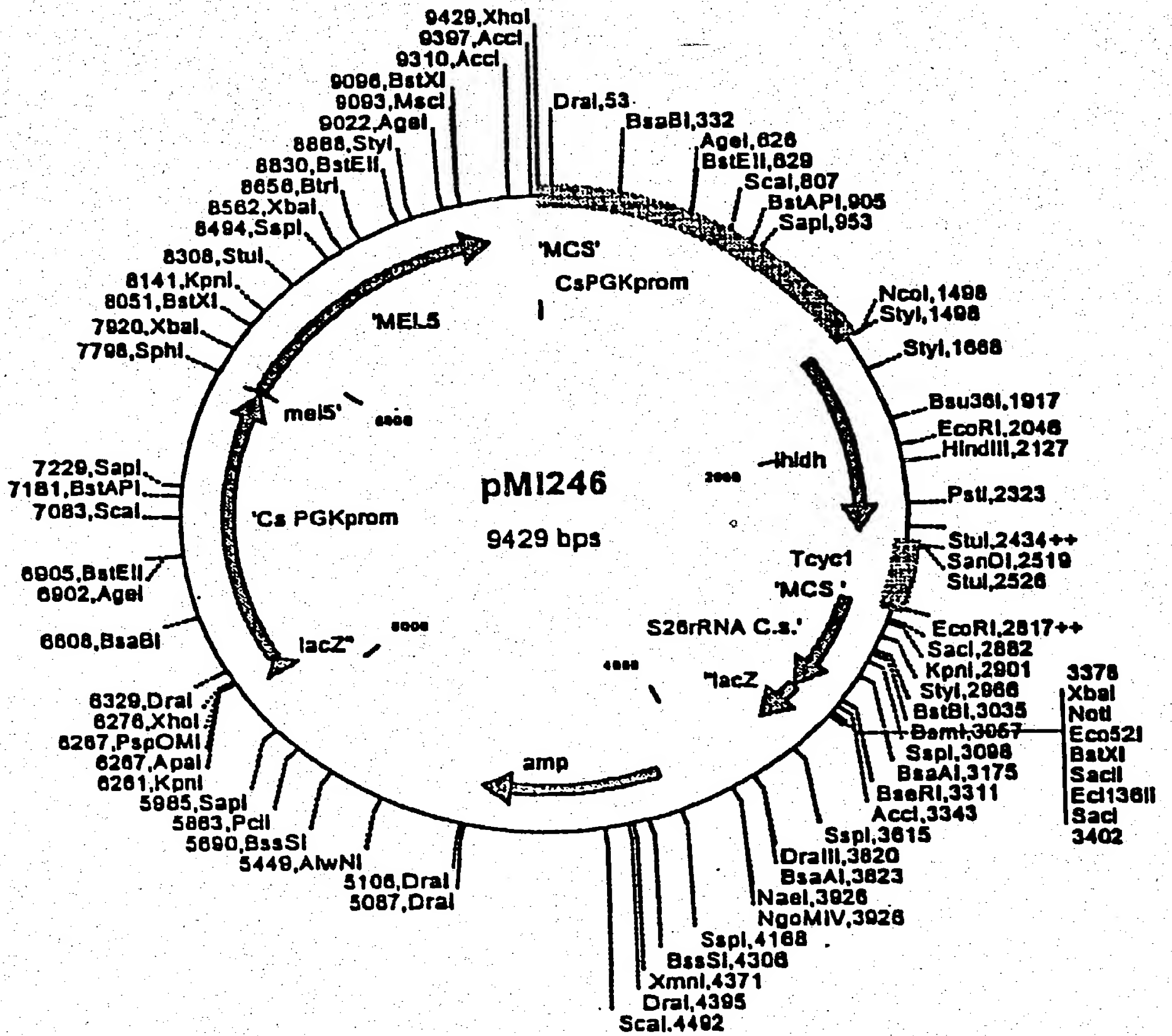
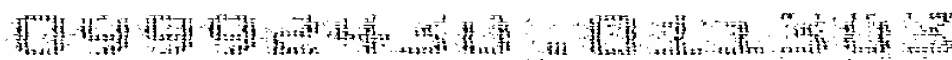


FIGURE 16 (10/11)





**pMI247**  
8461 bps

Genetic elements and features:

- amp<sup>r</sup>**: Ampicillin resistance gene.
- lacZ**: LacZ reporter gene.
- S28rRNA C.s.**: 28S ribosomal RNA coding sequence.
- 'MCS'**: Multiple cloning site.
- Tcyc1**: Tetacycline resistance gene.
- ihdh**: Isopentenyl diphosphate hydroxylase gene.
- CsPGKprom**: Constitutive promoter from *Candida*.
- MEL5**: MEL5 gene.
- 'Ca TDHprom**: Constitutive promoter from *Candida*.
- mel5'**: MEL5 gene 5' end.

Restriction enzyme sites (clockwise from top):

- NaeI, 129
- NgoMIV, 129
- BsaAI, 232
- DraIII, 232
- SapI, 440
- AccI, 712
- BseRI, 744
- BsaAI, 880
- SapI, 957
- BamHI, 998
- BstBI, 1020
- Acc65I, 1154
- KpnI, 1154
- Ecl136II, 1173++
- BamHI, 1228++
- BsrGI, 1328
- StuI, 1529
- SanDI, 1535++
- StuI, 1621
- PstI, 1732
- BbsI, 1922
- HindIII, 1928
- EcoRI, 2009
- BbsI, 2092
- Bau36I, 2137
- HpaI, 2270
- NcoI, 2557
- Bpu1102I, 2836
- SapI, 3101
- BstAPI, 3145
- HpaI, 3231
- Scal, 3248
- BpmI, 3317
- AgeI, 3429
- BsaBI, 3719
- BspMI, 3886
- HpaI, 4023
- XhoI, 4055
- AccI, 4087
- AccI, 4174
- 4382, BstXI
- 4238, BspHI
- 4391, MscI
- 4462, AgeI
- 4730, BpmI
- 4828, BtrI
- 4922, XbaI
- 4954, BspMI
- 4990, SspI
- 5176, StuI
- 5343, Acc65I
- 5343, KpnI
- 5408, BspMI
- 5427, BstXI
- 5564, XbaI
- 5686, SphI
- 5766, PacI
- 5791, PacI
- 5821, SspI
- 6041, BstWI
- 6128, BsaAI
- 6255, KpnI
- 6530, SapI
- 6553, PciI
- 6826, BsaSI
- 7064, AhaNI
- 7373, BspHI
- 7631, BpmI
- 8024, Scal
- 8141, XmnI
- 8210, BsaSI
- 8348, SspI
- 8381, BspHI



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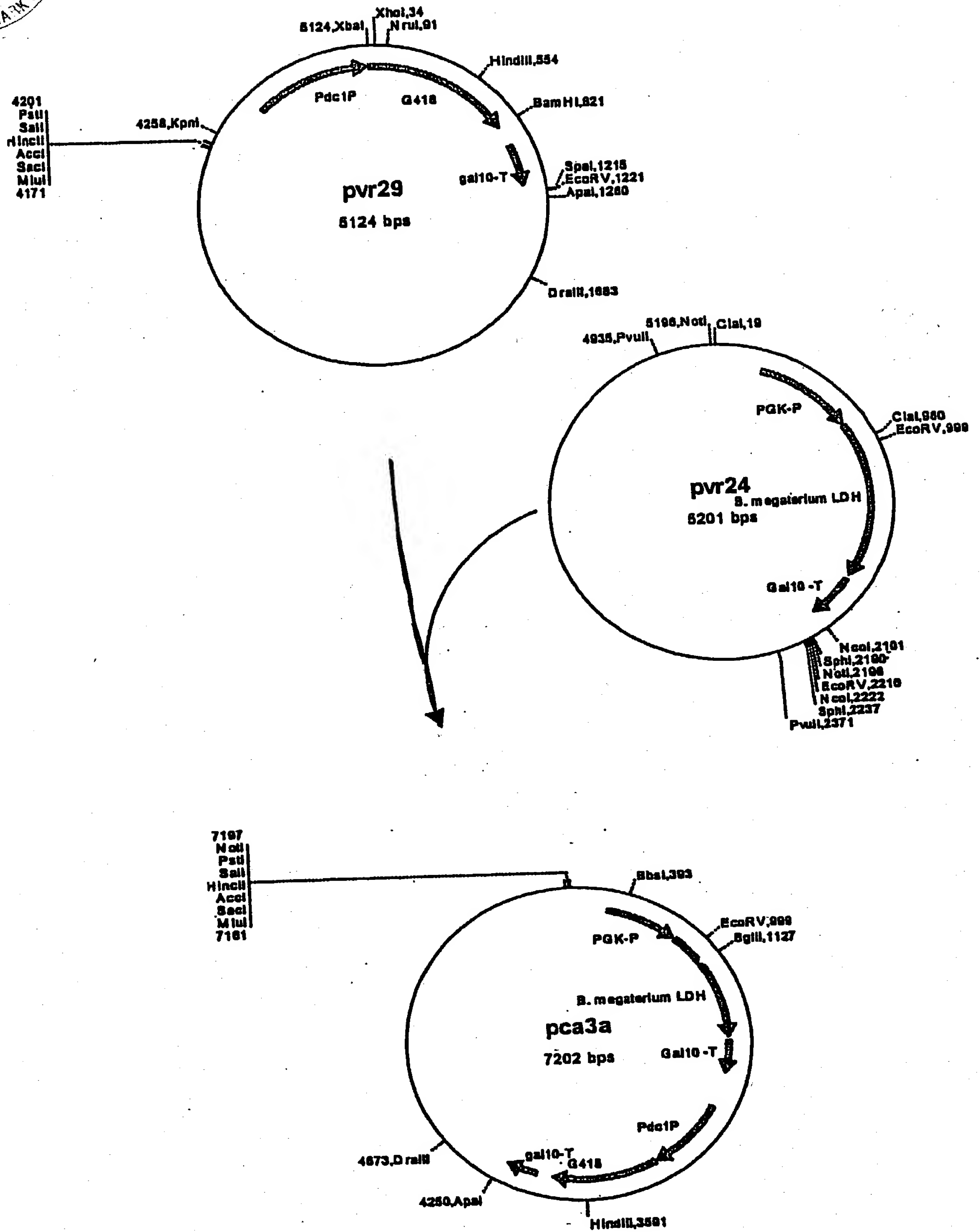
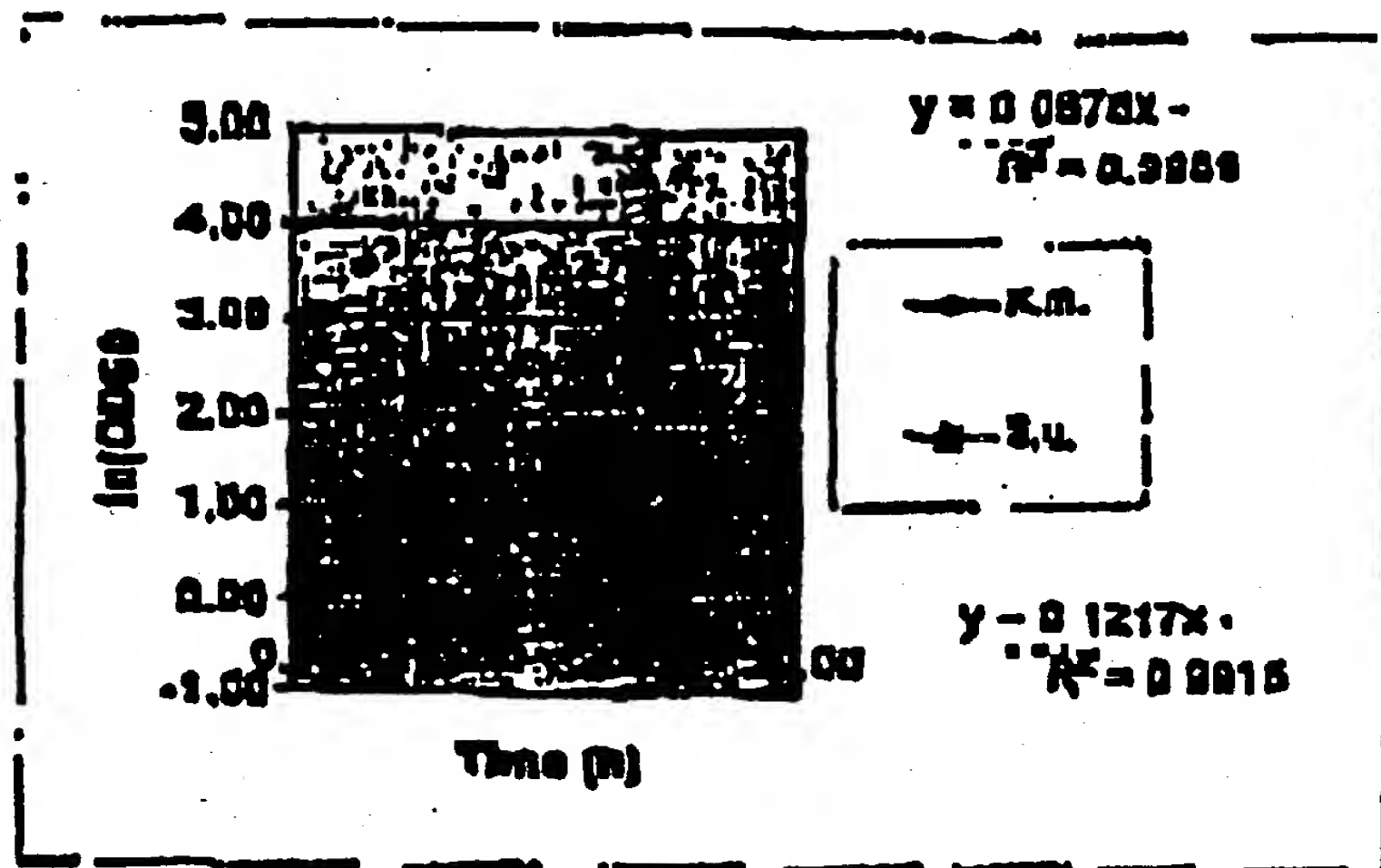


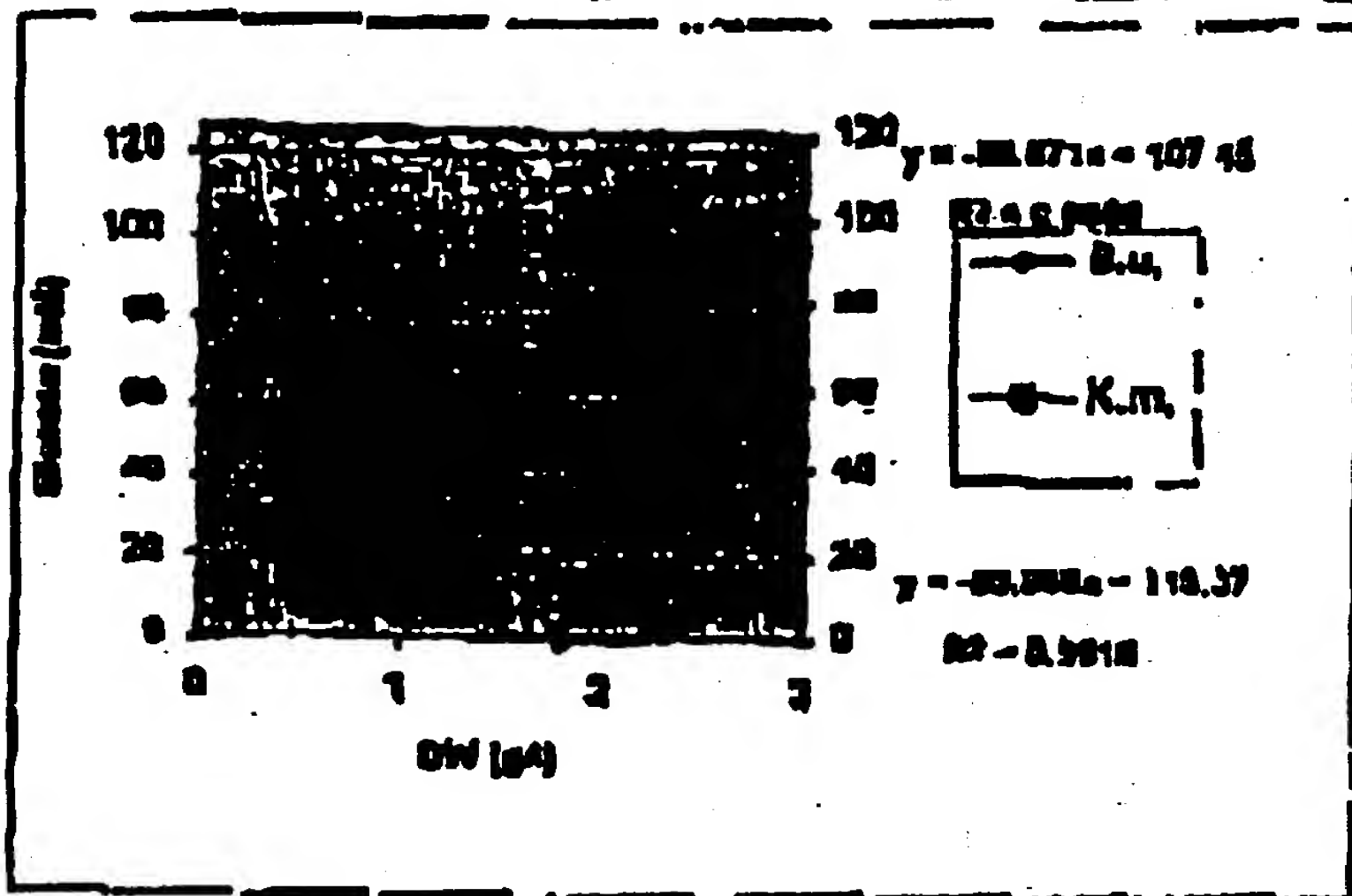
FIGURE 17



A



B



C

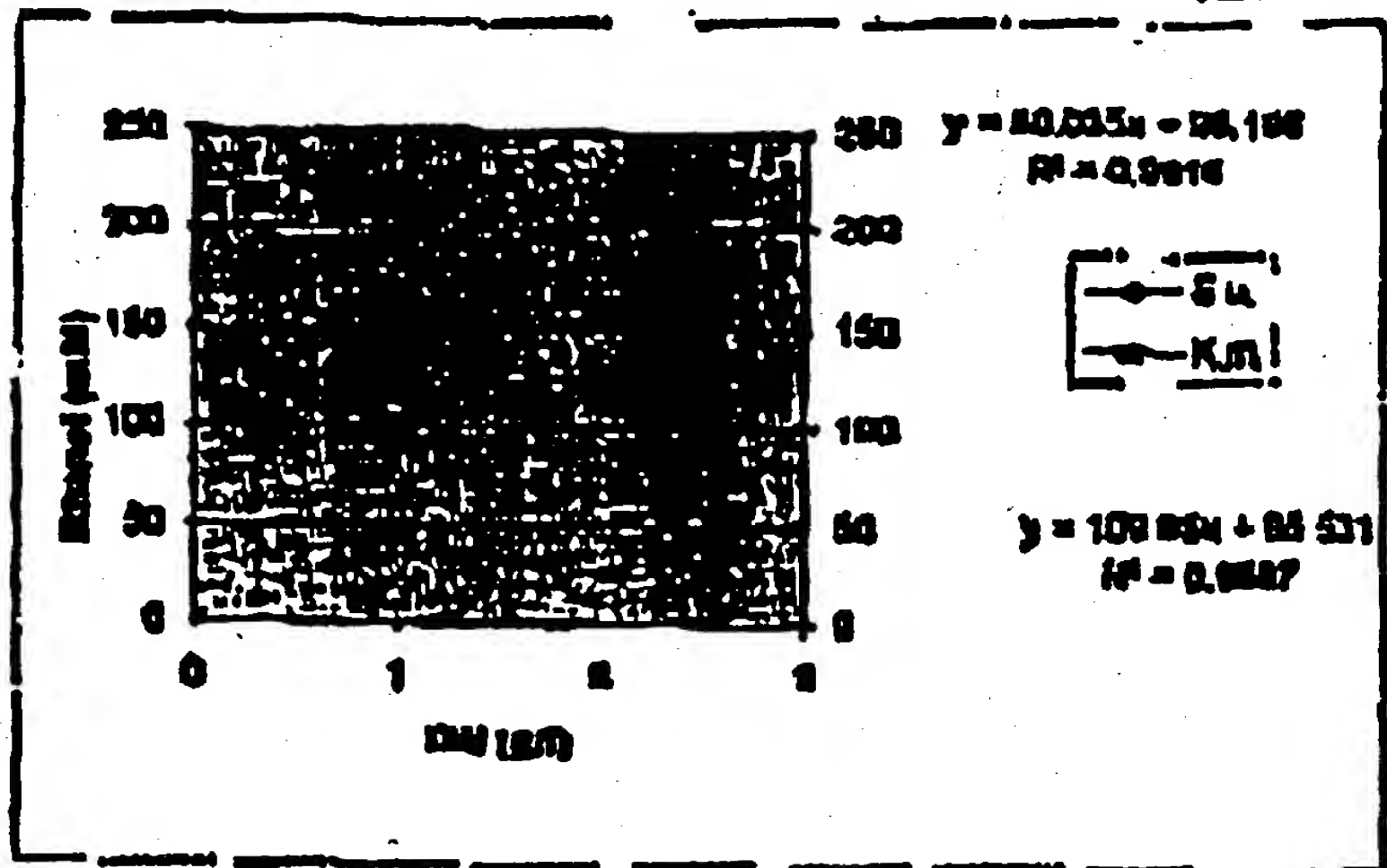
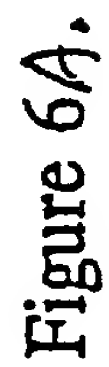
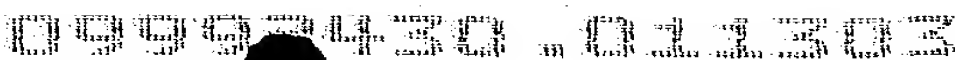


FIGURE 13





**Generic PDC Knock-out Fragment (2068 bps)**